

CHILDHOOD OBESITY: CAUSES AND CONSEQUENCES

PhD Thesis

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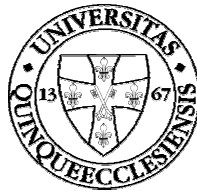
Department of Paediatrics, Medical Faculty, University of Pécs

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INTRODUCTION

An alarming rise in overweight and obesity was observed worldwide, particularly during the 1990's, in both the developed and developing countries. In Hungary, the prevalence of obesity increased from 12% to 16 % between 1980' and 1990's among schoolchildren. Obesity is a serious health risk; it is a major determinant of different diseases such as diabetes mellitus, hypertension, heart and kidney failure, atherosclerosis, cancer, infertility, birth complications and arthritis. On the other hand, obesity is largely preventable through changes in lifestyle.

Obesity is a heterogeneous group of conditions with multiple causes. Body weight is determined by an interaction between genetic, environmental and psychosocial factors.

Obesity is one of the features accompanying numerous genetic syndromes, like Prader-Willi (PWS), Cohen, Alstrom and Bardet-Biedl syndrome that have been genetically mapped. The first gene identification in obese human subjects was linked with the screening for genes identified previously in rodent models of monogenic obesity. However, the genetic susceptibility to obesity is in most cases polygenic, and is rarely the result of a Mendelian gene (monogenic obesity). Apart from rare obesity-associated syndromes, the genetic influences seem to operate through susceptibility genes. One of the two approaches to identify susceptibility genes is the candidate gene approach. It involves testing the association between obesity and a specific allele of a gene either in a family study or in a large cohort of unrelated controls and patients. Several candidate genes have been associated with human obesity and its metabolic complications, which include e.g. receptors that are important in thermogenesis (β 3-adrenergic receptor /3-BAR / gene and the family of uncoupling proteins), as well as those involved in appetite regulation.

The effects of catecholamine are modulated through four subtypes of adrenoceptors. The human 3-BAR is expressed predominantly in visceral fats, where it plays a role in determining the resting metabolic rate through its ability to stimulate lipolysis and thermogenesis. The involvement of 3-BAR in energy metabolism originates from observations indicating the prevalence of the genetic variant at the codon 64 of the 3-BAR gene leads to replacement of a tryptophan with an arginine (Trp64Arg). This polymorphism has been reported to be associated with abdominal obesity, a propensity for weight gain, high BMI, insulin resistance and an earlier onset of type 2 diabetes mellitus. Other studies, however, could not demonstrate these associations.

An obese child will remain obese in adulthood and become at risk for acquiring or increasing coronary heart disease in 30 % to 60 % of cases. Although genetics may have a part of it,

several environmental factors affect this phenomenon significantly. The first contact of the child with the environment is in the uterus. Over- or under-feeding in pregnancy has been associated with the development of obesity in later life. Beside of the prenatal period, the other critical period is the adolescence in the development and persistence of overweight in the paediatric age group. The period of 'adiposity rebound' may constitute a third. It is when the BMI begins to increase after reaching a nadir in early childhood. Small for gestational age (SGA) has been associated with increased risk of diseases such as diabetes or cardiovascular disease in adulthood. Infants with high birth weights appear to have an increased risk of subsequent overweight.

The presence of inherited and acquired thrombophilias has recently been linked to most cases of maternal venous thrombotic events as well as these adverse obstetric outcomes. One of the most common inherited thrombophilias is heterozygosity for the factor V Leiden mutation. Although there is no consensus on the association between the factor V Leiden mutation and early pregnancy loss, but the evidence suggests an association between the mutation and late (first-, second-, third-trimester) fetal loss, severe preeclampsia, abruption and severe intrauterine growth retardation (IUGR).

Over the past two decades, many papers have demonstrated in the literature that low birth-weight, thinness and short body length at birth are associated with increased risk of atherosclerosis, type 2 diabetes mellitus (T2DM), hypertension and metabolic syndrome. This observation, which originated at Hertfordshire in the United Kingdom, was confirmed in other countries such as Sweden and Netherlands. The association of low birth-weight with adult cardiovascular disease led to the 'fetal origins or thrifty phenotype hypothesis' formulated by Barker.

Obese children have a higher prevalence of T2DM and insulin resistance and the frequency of this complication appears to have risen in recent years paralleling the worldwide increase in obesity in this age group. On the basis of the available data, the prevalence of T2DM in Caucasian children and adolescents seems to be much lower than those reported in other races, but more representative, population-based surveys are needed. In April 2002, a questionnaire was distributed among European Childhood Obesity Group (ECOG) representatives from 16 European countries, which included several questions concerning the prevalence, risk factors and complications of childhood obesity, such as T2DM. From nine countries, altogether 184 European children were diagnosed with T2DM, 144 of them of Caucasian origin. The majority of them were overweight females and had positive family history for T2DM. Although general principles of treatment of T2DM in adolescents are

similar to the treatment of adults there is general agreement between the paediatricians dealing with this problem that they should not be a simple extrapolation. Treatment strategies should be based on symptoms at presentation. Asymptomatic children identified at routine testing should be counselled on the necessary lifestyle changes. Therapeutic strategies include lifestyle and behaviour modification, nutrition education, and psychological and family therapy interventions. Because obesity is the major problem in most adolescents with T2DM, dietetic advice is mandatory, although calorie intake should not be too restricted to ensure normal growth and pubertal development. Patients should be encouraged to increase their physical activity or at least to decrease inactivity.

Childhood onset of adult cardiovascular disease has become a significant public health problem that needs to be addressed globally and individually. Whether genetic, environmental, or fetal influences are the primary culprits in the epidemic of obesity-related adult cardiovascular diseases seen today remains unknown. In spite of this, the interventional focus should be placed on early life, and health care providers and public health professionals should pay attention to the elevated future coronary heart disease risk among children. Better understanding of the aetiology of these diseases hopefully will lead to more effective, targeted preventive measures and therapy.

AIMS

1. Trp64Arg polymorphism of the β_3 -adrenergic receptor gene

1.1 To examine the frequency of Arg64 allele of the β_3 -adrenergic receptor (3-BAR) gene, which is one of the known candidate genes, in healthy and obese Hungarian children.

1.2 To look for possible associations between this polymorphism and some clinical and metabolic characteristics of obese children.

2. Leiden mutation; size at birth and later risk factors

2.1 To test the prevalence of Leiden mutation in the mothers of premature infants and in the mothers of intrauterine-growth-retarded children.

2.2 To determine the association between size at birth and later risk factors (hypertension, hyperinsulinism, hyperglycaemia, dyslipidaemia) in prepubertal children.

3. Impaired glucose tolerance and type 2 diabetes mellitus

3.1 To examine the prevalence of impaired glucose tolerance (IGT) and T2DM in obese Hungarian children.

3.2 To assess the effects of a 6-months diet and life-style changes in the children with IGT and T2DM.

SUBJECTS

Written informed consents were obtained from the subjects and all parents of the children before enrolment in the different studies. All of the studies were approved by the ethic review committee of the University of Pécs.

1.1 In all, 295 obese children (male:168) were included in the study after the exclusion of any endocrinological disorder, nutritional-, growth- and renal problems or obesity syndromes. A total of 147 healthy, non-obese children (male: 68) recruited from elementary schools, served as controls. The average age of the children in the two groups was 12.6 ± 3.2 and 12.4 ± 1.7 , respectively.

1.2 Obese children carrying the Arg64 allele (n=35, male: 23) were compared to randomly chosen, obese children without the Arg64 allele (n=35, male: 20).

2.1 White (Caucasian) mothers of premature (Group PM; n=50) and mothers of intrauterine growth retarded neonates (Group IUGR; n=56) were tested. The newborns were considered as premature when their gestational age was < 37 weeks. Intrauterine growth retarded children were born full term with birth weight, height and head circumference below the 10th centile (proportional) or with birth weight below the 10th centile, but with normal length and head circumference (disproportional).

2.2 229 children (134 boys, 95 girls) were examined at the age of 6-10. We compared children born full term with normal weight, height and head circumference (1st group), the children born full term with birth weight, height and head circumference less than 10th centile (2nd group), children born full term with birth weight less than 10th centile and with normal length and head circumference (3rd group) and children who were preterm at birth (4th group). The age of children at the time of investigation was comparable in the four groups.

3.1-2 Oral glucose tolerance test (OGTT) was performed in 289 obese (153 boys) (mean BMI \pm SD: 31.1 ± 4.6 kg/m²) adolescents (mean age \pm SD, 12.9 ± 2.7 years). After 6 months, the OGTT was repeated in children with IGT and DM.

METHODS

Anthropometric measurements

Weight was obtained with subjects wearing light clothing to the nearest 0.1 kg on a standard beam scale. Height was measured to the nearest 0.1 cm by a Holtain stadiometer. In all examinations, children were considered as obese if their body weight exceeded the expected weight for height with more than 20%, and if body fat content with more than 25% in males and 30% in females. BMI was calculated according to the formula, real weight (kg)/height²(m²), while body fat (BF) was estimated from skinfold measurements, which were performed with a Holtain caliper, using Parizkova and Roth's formula. The body weight of control, healthy children was less than 120% of the expected weight for height.

Blood pressure measurements

Blood pressure (BP) was measured using a Mercury sphygmomanometer with proper cuff size in standard conditions. 3-5 occasional BP values were obtained and if the average of the blood pressure values was above the 95th centile for age and sex, 24 h ambulatory blood pressure monitoring (ABPM) was performed with a non-invasive recorder (Meditech, Hungary) using oscillometric method. Those children whose mean 24h arterial blood pressure value exceeded the 95th centile value for height and sex were considered hypertensive. (In the study of prepubertal children who were intrauterine growth retarded neonates, there was no possibility to perform the ABPM, so we considered children to be hypertensive when the lowest blood pressure value of the three measurements was above the 95th centile for age and sex.)

Laboratory tests

Determination of the Trp64Arg polymorphism in exon 1 of the 3-BAR gene

DNA was prepared from peripheral blood leukocytes by salting out procedure. Exon 1 was amplified with polymerase chain reaction (PCR) using the primers BstN UP: 5'-CGCCAATACCGCCAACAG-3' and BstN DOWN: 5'-CCACCAGGAGTCCCATCACC-3' (product size 210 bp). Restriction enzyme digestion products were separated on a 3% agarose gel and visualized by staining with ethidium bromide.

Determination of factor V Leiden mutation

The factor V Leiden mutation was tested from dried blood-spot samples according to Zöller and Dahlbäck, by PCR method. The sequences of the PCR primers (Ransom Hill Bioscience, Ramona, CA) for the amplification were the following: forward primer: 5'GGGCTAATAGGACTACTTCTAATC3'; reverse primer: 5'TCTCTTGAAGGAAATGCCCATTA3'. The DNA was digested by MnlI restricted enzyme (Stratagene). The resulting PCR products were separated by electrophoresis on a 2.2 % agarose gel with ethidium-bromide staining.

Biochemical studies

Plasma glucose, serum insulin and lipid levels were determined from blood samples taken after an overnight fast in obese children. In obese children OGTT (1.75 g/kg ideal body weight, max. 75 g) was performed. Plasma glucose and serum insulin levels were determined at 0 and 120 min at the OGTT by the glucose oxidase method and by commercially available radioimmunoassay (RIA) kits, respectively. The criteria of impaired glucose tolerance and diabetes mellitus were based on the recommendation of American Diabetes Association. Insulin resistance was estimated by the Homeostasis Model Assessment (HOMA) using the formula: fasting serum insulin ($\mu\text{IU/ml}$) x fasting plasma glucose (mmol/l)/22.5. (In the study of prepubertal children who were intrauterine growth retarded neonates, the cut-off value for fasting insulin was 20 $\mu\text{IU/ml}$, while hyperglycaemia was considered when the fasting blood glucose level was more than 6.2 mmol/l .)

Serum cholesterol and triglyceride levels were determined by the enzymatic method with Boehringer kits; serum high-density lipoprotein (HDL) cholesterol was measured according to the method of Steele et al.. Serum cholesterol, triglyceride and HDL-cholesterol were considered high or low when they fell above or below the recommended values of the Hungarian Lipid Consensus Conference: serum cholesterol > 5.2, HDL-cholesterol < 0.9 mmol/l and serum triglyceride > 1.1 mmol/l (< 10 years)- > 1.5 mmol/l (> 10 years). If the value of any of these parameters was abnormal, the child was considered dyslipidaemic.

Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 7.5, 8.0 and 10.0. Data are presented as means \pm SD. Statistical significance of the differences between groups was evaluated using the Fisher's exact or Chi-square or Student's t-test or ANOVA, when appropriate.

RESULTS

1. Trp64Arg polymorphism of the 3-BAR gene

(8, 11, 13, 14; abstr: 2,6)*

1.1 The frequency of Trp64Arg polymorphism in normal and obese Hungarian children was similar. The mutation occurred in 14 healthy (male: 7) and 35 obese children (male: 23), of whom 2 were Arg64Arg homozygote and 33 were Trp64Arg heterozygote.

1.2 The obese children with Arg64 allele were compared to a group of obese children without it. The latter group was formed by a computer-generated randomisation. The anthropometric data of obese children with and without polymorphism are shown in Table 1.

Table 1. Anthropometric data of obese children with and without polymorphism (mean±SD)

	Trp64Trp (n=35) (male: 20)	Trp64Arg/Arg64Arg (n=33+2) (male: 23)
Age (years)	12.3 ± 2.9	12.6 ± 2.9
Body height (cm)	155.7 ± 15.9	161.4 ± 15.4
Body weight (kg)	75.6 ± 17.7	81.2 ± 23.2 **
BMI (kg/m²)	30.9 ± 3.9	35.0 ± 10.9 ***
BF (%)	36.5 ± 2.3	38.8 ± 3.9 ***
Systolic blood pressure (mmHg)	114.5 ± 8.3	125.2 ± 10.1 *
Diastolic blood pressure (mmHg)	72.5 ± 9.0	73.2 ± 8.4

*** $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ *BMI: body mass index; BF: body fat*

The weight of obese children with Arg64 allele was significantly higher ($p < 0.01$) than those without the polymorphism. Similar tendency ($p < 0.05$) was observed in the BMI and BF values.

*Numbers in parenthesis are serial numbers of the papers and abstracts which were written in the issue of the thesis.

The serum insulin levels and HOMA were significantly higher in children carriers of Arg64 allele as compared to those not having this. Since the two groups were significantly different in respect of BF and BW, and these factors are closely related to insulin levels and HOMA index, therefore these latter two parameters were corrected for BW and BF. The corrected values remained significantly different between the two groups. Serum triglyceride, total cholesterol and HDL-cholesterol levels were not different between the two groups. Systolic BP of subjects with Trp64Arg and/or Arg64Arg genotype was also significantly higher ($p<0.001$) than that of those with the Trp64Trp genotype (Table 1.).

2. Leiden mutation; size at birth and later risk factors
(3,4,5)

2.1 The prevalence of the Leiden mutation in an apparently healthy Hungarian Caucasian population sample of our region was 6.33 %, which was comparable with other European prevalence rates. In the group with IUGR the prevalence of heterozygosity was not significantly different from that of the healthy Hungarian population, while in the preterm the prevalence was 18 %. As compared to the 6.3% prevalence rate of the healthy Hungarian population, this 18 % value of the mothers of premature neonates proved to be significantly higher ($p<0.01$). The difference between Groups PM and IUGR (18% versus 7.2%) was also significant statistically (Table 2.).

Table 2. Prevalence of factor V Leiden mutation in the two groups

	Normal (% of total)	Heterozygotes (% of total)	Homozygotes (% of total)
Group PM (n=50) (mothers of prematures)	41 (82)	9 (18)	-
Group IUGR (n=56) (mothers of intrauterine growth retarded neonates)	52 (92.8)	3 (5.4)	1 (1.8)

Figures in parenthesis indicate percent of total.

2.2 According to the results cardiovascular risk factors cannot be found among children at the age of 6-10 who were born with low birth weight. The anthropometric data of children are shown in Table 3.

Table 3. Anthropometric data of children at the time of examination (prepubertal stage) (mean±SD)

	Full term neonates			Premature
	normal (n=24)	proportional IUGR (n=90)	disproportional IUGR (n=25)	(n=90)
	1. group	2. group	3. group	4. group
Age (years)	8.8±1.5	8.1±1.5	8.7±1.7	9.2±1.6
BW (kg)	32.2±7.4	24.9±7.2* **	28.0±8.6	30.1±9.2
BH (cm)	134.4±10.1	122.6±9.3 **	128.2±9.9	130.3±8.3
BF (%)	22.9±6.8	20.5±5.9	20.2±4.4 **	20.9±6.4
LBM (kg)	24.4±4.2	19.6±4.6* **	22.2±6.7 **	23.4±6.2

* $p < 0.01$, ** $p < 0.001$

BW: body weight, BH: body height, BF: body fat; LBM: lean body mass

BW: $p < 0.001$ 1. vs. 2. group; , $p < 0.01$ 2. vs. 4 group

BH: $p < 0.001$ 1. vs. 2. 2. vs. 4 group

BF: $p < 0.001$ 1. vs. 3. group;

LBM: $p < 0.001$ 1. vs. 2., 3. group, $p < 0.01$ 2. vs 4 group

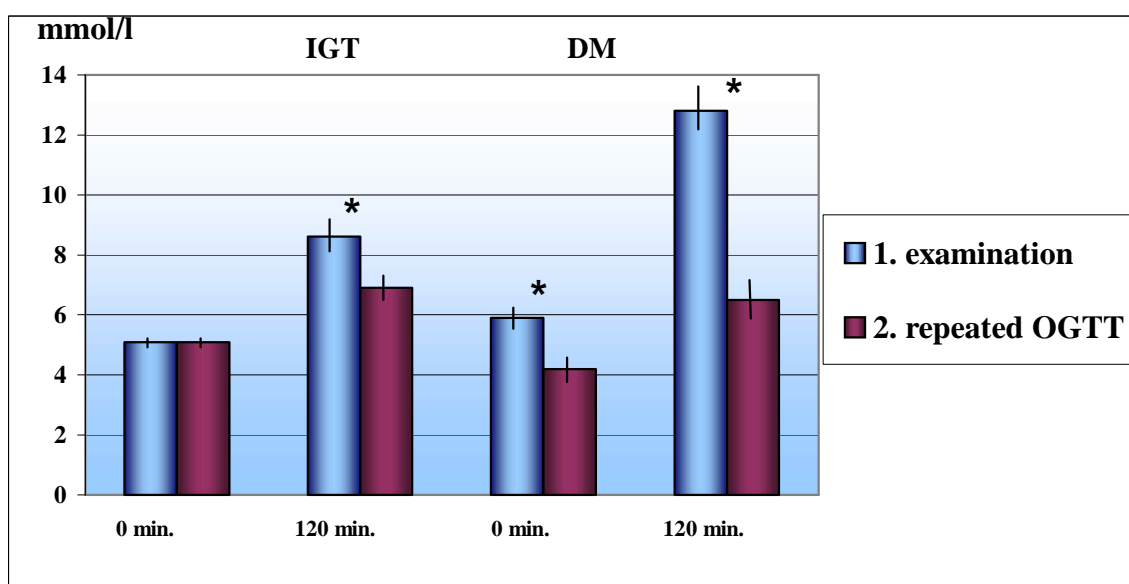
Weight and height of the children in the 2nd group were significantly lower than in the 1st and 4th groups (2nd group vs. 4th group : $p < 0.01$; 2nd group vs. 1st group: $p < 0.001$). The laboratory results were normal. Dyslipidaemia was found 21% in the 1st group, 17 % in the 2nd group, 16% in the 3rd group and 28% in premature. There was no significant difference among the four groups. The mean of the systolic and diastolic blood pressures were similar in the four groups. Hypertension was detected in 12.5 % of the 1st and 3rd groups, in 5.6 % of the 2nd group and in 8.9 % of the 4th group.

3. Impaired glucose tolerance and type 2 diabetes mellitus

(1,2,6,7,9,10,12; abstr: 1,3,4,5,7)

3.1 Because of the scarce European, especially Hungarian data of the prevalence of T2DM our aim was to evaluate the frequency of IGT and T2DM among clinically healthy, obese children. IGT was found in 50 children (17.3 %), while the prevalence of T2DM was 1.7 % ($n=5$), so altogether the disorders of carbohydrate metabolism could be detected in 19.0 % of the children ($n=55$).

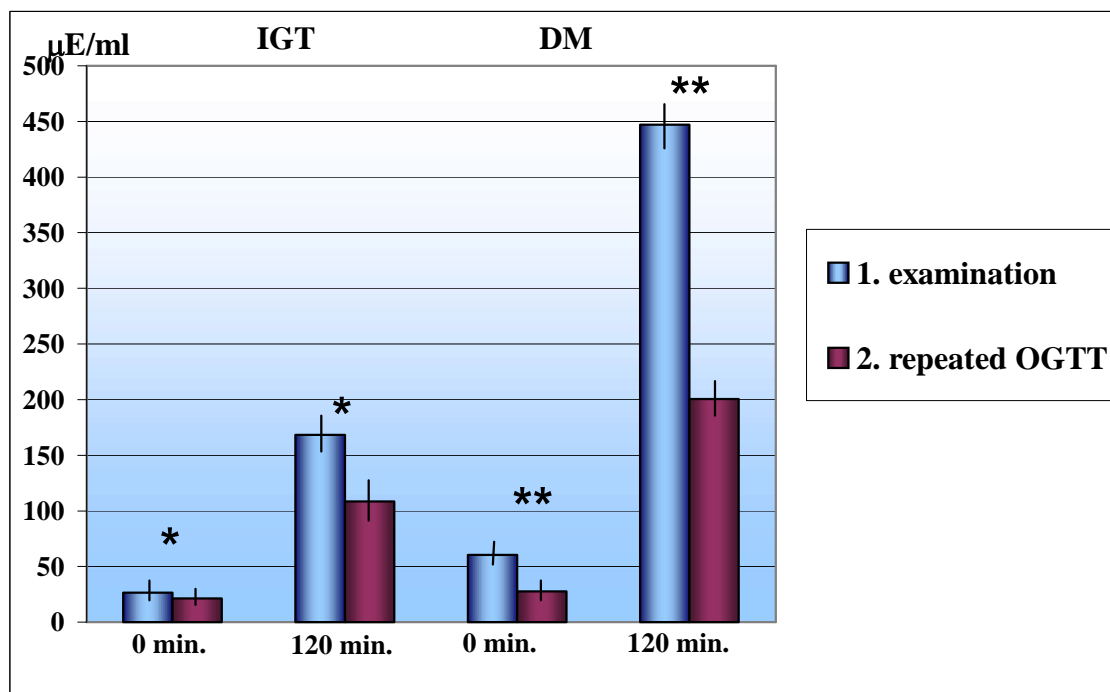
The children with disturbed carbohydrate metabolism, a low calorie (1500 kcal/day), carbohydrate (200-250 g/day) diet, and regular exercise were recommended and they were called back for a repeated OGTT after six months.



* $p < 0.001$

Figure 1. Changes of blood glucose (mean±SD) after a 6-months diet and exercise (n=36)

3.2 32 children with IGT and 4 children with T2DM took part in the repeated OGTT. Although the body weight was not changed significantly, BMI decreased significantly (30.4 ± 4.9 vs 29.0 ± 4.4 kg/m²; $p < 0.05$). The changes of mean blood glucose and serum insulin levels are shown in Figure 1 and 2.. HOMA index also decreased significantly (6.7 ± 3.7 vs 4.9 ± 3.3).



* $p < 0.001$ ** $p < 0.05$

Figure 2. Changes of serum insulin levels (mean±SD) after 6-months diet and exercise (n=36)

OBSERVATIONS AND PRACTICAL CONSEQUENCES OF THE STUDIES

1. Trp64Arg polymorphism of the 3-BAR gene

- The frequency of Trp64Arg polymorphism was similar in Hungary as compared to other European countries, and there was no difference between healthy and obese children, however the possession of Arg64 allele in obese children is associated with higher degree of obesity, insulin resistance and hypertension, but the number of cases need to be increased and further studies are needed to clarify these associations.
- To the best of my knowledge, this is the first study of children in Hungary investigating the frequency of a candidate gene of obesity and its effect on cardiovascular risk factors.
- Although, in the literature, there are controversial results of the role, not only, of Trp64Arg polymorphism of 3-BAR gene, but other candidate genes, in developing obesity, according to our findings and to know that obesity a multifactorial and heterogeneous disorder, therefore, preventive measures for obesity, to be fully effective in a population, must based on the modification of several potential risk factors simultaneously. Comprehensive, successful prevention programs are needed which should focus on promoting and supporting healthful lifestyles for all children at home, in school and in the society.
- Although the genetic analyses of the samples started at the Department of Clinical Genetics and Child Development of University of Pécs, these investigations made the possibility to create a scientific PCR laboratory at the Department of Paediatrics of University of Pécs.

2. Leiden mutation; size at birth and later risk factors

- Our results are in concert with the findings of some studies that the presence of Factor V, Leiden mutation may have a role in premature delivery.
- According to our results, cardiovascular risk factors cannot be proved among children at the age of 6-10 who were born as intrauterine growth retarded. Further studies are required to determine whether which stage of pregnancy might influence birth weight and later risk factors, and it can be important at what age of children should be examined for these risk factors.

3. Impaired glucose tolerance and type 2 diabetes mellitus

- According to our prevalence data of T2DM, it seems that not it is the major problem among obese youth, in spite of this, clinically healthy obese children have disturbances of carbohydrate metabolism, so screening for T2DM in children and adolescents, as suggested by the American Diabetes Association, is highly recommended.
- Impaired glucose tolerance and T2DM in asymptomatic obese children can be managed with dietary and lifestyle interventions, resulting improvement of the metabolic status of these children.
- According to this observation and assuming that T2DM is preventable, there are two components of the primary prevention. First of all, a populations strategy is needed, for altering the lifestyle and the environmental determinants of T2DM. Second, a high-risk strategy is needed for screening individuals at especially high risk for T2DM and bringing preventive care to them.

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LECTURES AND POSTERS IN THE ISSUE OF THE THESIS

1. A Magyar Elhízásellenes Alapítvány VII. Konferenciája
1996. szeptember 18-22., Balatonlelle
Erhardt É., Molnár D.: *Intrauterin tápláltság szerepe a felnőttkori elhízásban és a kardiovaszkuláris betegségekben*
2. 7th International Workshop of European Childhood Obesity Group
21-22nd November, 1997, Verona, Italy
E. Erhardt, D. Molnár: *Size at birth and later risk factors*
3. A Magyar Elhízásellenes Alapítvány XII. Konferenciája
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10. Magyar Diabetes Társaság XVI. Kongresszusa
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11. 28th Annual Meeting of the International Society for Pediatric and Adolescent Diabetes (ISPAD)
18-21 September 2002, Graz, Austria
E. Erhardt, D. Molnár, Gy. Soltész: *Impaired glucose tolerance and type 2 diabetes in obese Hungarian children.*
12. 12th European Congress on Obesity
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13. A Magyar Gyermekorvosok Társaságának 2003. évi Nagygyűlése
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15. Magyar Gyermekdiabetológiai szekció Tudományos Ülése
2003. 10.17.-18., Szeged
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17. 13th European Congress on Obesity
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E. Erhardt E., K. Csernus, M. Czakó, D. Molnár: Frequencies of single-nucleotide polymorphisms of some candidate genes playing role in thermogenesis in Hungarian children (poster).
18. XIV. Symposium of Polish Pediatric Endocrinology
15-17 October, 2004, Wisla, Poland
E. Erhardt: Is type 2 diabetes mellitus a significant problem in European children? (felkért, plenáris előadás)
19. 14th European Congress on Obesity
1-4 June, 2005, Athen, Greece
E. Erhardt, K. Csernus, D. Molnár: Examination of synergetic effects of some candidate genes playing role in thermogenesis (poster).
20. ECOG International Workshop
29 Sept-1 Oct, 2005, Vienna, Austria
E. Erhardt, K. Csernus, Sz. Bokor, D. Molnár: Frequency and effect of Ala 12 allele of PPAR γ on cardiovascular risk factors in Hungarian children (poster)
21. Obezitológiai szimpózium és továbbképző tanfolyam
2005. október, Pécs
Erhardt É: A gyermekkori elhízás helyzete és kezelése (felkért előadás)
22. Gyermekkori Diabetes és Obesitas Továbbképző Tanfolyam
2006. március, Pécs
Erhardt É: 2-es típusú diabetes mellitus (felkért előadás)
23. X. Családorvosi konferencia
2007.10. 06., Budapest
Erhardt Éva: Az elhízás genetikája (felkért előadás)

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